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PHAGOCYTIC ACTIVITY OF BLOOD NEUTROPHILS IN TULAREMIA IN THE ANIMALS WITH DIFFERENT INFECTIOUS SENSITIVITY

T. N. Dunaeva, K. N. Shlygina

A study was made of the ingestive capacity of blood neutrophils; there were revealed no significant differences in the intact animals with a different infectious sensitivity to tularemia. With the development of infection the ingestive activity of leukocytes increased in the infected highly sensitive animals, but the digestive function was not manifest. In albino rats (with a low sensitivity to tularemia) the disease induced an intensification of the ingestive and the manifestation of the digestive function of neutrophils dynamically developing together with the specific immunity reactions.

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PHAGOCYTIC ACTIVITY OF NEUTROPHILS IN TULAREMIA IN ANIMALS WITH VARYING INFECTIOUS SENSITIVITY

Article by T. N. Dunayeva and K. N. Shlygina, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciencies, Moscow, submitted 4 November 1974, with tularemia, we note a rise in the absorption activity of neutrophils among vaccinated or previously ill persons and laboratory animals /1, 3, 4, 11/. A study of tissue culture showed that the digestive activity of peritoneal monocytes and polynucleat leucocytes /12, 13/ increases among immune guinea pigs and rabits.

Our study encompasses a comparative evaluation of the absorptive and digestive ability of polymorphnuclear leucocytes relative to the tularemia microbe in animals with varying infectious sensitivity. We investigated the opsonocytophagic reaction (OCR) among untreated animals and animals infected in the dynamics of the infection process. The methodology for setting up the OCR was standard: a specific volume of citrate blood was mixed with a live virulent culture thereby obtaining in the suspension 500

million bacteria in 1 ml. The fixed smears were stained with the Romanovskiy-Gimza stain diluted with acetone /7/. We calculated the OCR absorption index in the smear according to the methodology accepted for brucellosis. Culmination of phagocytosis was calculated relative calculated after 30 minutes. Am increase in the quantity of bacteria in neutrophiles, as well as an increase in phagocytic cells pointed to weak digestive ability of leucocytes. /2/. The OCR intensity was calculated among untreated animals: albinomice, common field mice, golden hamsters, and guinea pigs, which are highly sessitive to tularemia, and albino rats and rabbits which are less sensitive.

Absorption of tularemia bacteria by polymorphnuclear leucocytes was found among all species of animals. After 30 minutes the OCR index varied in different experiments regardless of the degree of infectious sensitivity of the animals to tularemia (Table 1). As the calculation of the digestive ability of neutrophiles showed the OCR index among the highly sensitive animals after 60 minutes was initially higher, on the average by 2 times or more, and among some albino mice and guinea pigs by 4 - 6 times. Among some guinea pigs the absorption index increased insignificantly, while among some animals it was even lower that the initial.

Among the less sensitive animals the absorption index after 60 minutes increased at a lower rate that among the higher sensitive animals. We observed, among many rabbits an expressed digestive ability of the leucocytes. The average indices relative to the index for individual experiments varied for the rabbits between 0.5 - 2.1 and for albino rats between 1.1 - 2.8.

The number of phagocytic cells after 30 minutes varied between 16 and 53% for albino mice, from 13 - 51% for guinea pigs, from 24 - 30% for albino rats, and 16 - 68% for rabbits. Calculations after 60 minutes showed that the amount of active neutrophiles increased among all highly sensitive animals and albino rats. Among some rabbits the percentage of phagocytic cells decreased or the increase was insignificant pointing to the bacteriocidal or bacteriostatic effect. Changes in the nature of OCR were studied 30 albino mice and 6 rats infected with the subcutaneous virus strain №2 503 in a dose of ?? microbe cells according to the optic standard of turbidity. We examined 4 animals each period. The albino mice were slaughtered, while blood of the albino rats was drawn while they were still alive, from the heart or tail. Reactions were established simultaneously for all animals studies from the first to the eighth days of the experiments, and for the rats during the

15 - 20th days folloeing infection.

The absorption index for a 30-days exposure of mice and rats increased naturally from the second to the sixth days. The mice died on the sixth to seventh days, among rats the absorption index decreased during subsequent daus and by the 15th. - 20th. days did not differ from the index of the intact animals. The digestive function of the neutrophiles was not pronunced on the first day following infection; the absorption index for the mice and rats after 60 minutes was greater than the initial by 2.6 - 2.9 times, which matches the indexes for intact anumals (Table 2). On subsequent days we noticed in the mice a chronographic increase in the absorption index and in the percentage of phagocytic cells. Consequently phagocytosis was not attained.

In studying blood smears of the mice we noted fixation of tularemia bacteria on small accumulations of thrombocytes. This interaction of blood plateletes and microbes increased from the first to the fifth days following infection and was weak among intact mice. On the sixth day of the disease thrombocyte accumulation decreases and nearly disappears among some animals. At the same time the smears showed an increase in the number of freely arranged bacteria, which were neither fixed to the thrombocytes nor in a state of attraction with leucocytes.

We notes degenerative changes in the neutrophiles -- vacuolization of the protoplasm, pycnosis, phagolysis. Moreover, we found in the destroyed cells vacuoli with undigested bacteria. By the fourth to fifth days of the disease the blood showed plasmocytes, and occasionally macrophages. Phagocytic monocytes are seen rately. Agglomerates of neutrophiles and lymphocytes against a background of the accumulation of blood plateletes and bacteria are found rarely among mice.

In contrast to mice, rats clearly show large scale completion of phagocytosis, both for calculation of index relationships, and reduction of the percentage of phagocytic neutrophiles with an increase in the duration of the reaction (see Table 2). By the second day following infection the OCR indexes, after 60 minutes and 2 hours, were only insignificantly higher than after 30 minutes. Subsequently, we noted a normal decrease in the index, as well as in the number of phagocytic neutrophiles with an increase in the duration of the reaction. This oncrease in the digestive activity of neutrophiles was maintained throughout the entire period of observation (up to the 20th day of the experiment) and was also manifest during a decrease in the absorption index. Thus, during a chronographic calculation of the OCR index we found qualitatively new features in the

interaction of the phagocytic cells and the stimulat, which cannot be seen during a single calculation of phagocytosis activity.

Microscopically the reaction of blood elements in the rats differs from the reaction in mice by the large accumulations of tularemia bacteria on the blood plateletes, more frequently visible agglomerations of these accumulations with neutrophiles and lymphocytes and monocytes attached to them. The latter phagocytize bacteria somewhat more frequently. In the neutrophiles we can clearly see digestion of bacteria, a portion of these show a pale stain. We find large quantities of lysining leucocytes, remains of destroyed cells show digestive vacuoli, normally empty or containing insignificant amounts of bacteria.

Phagolysis of neutrophiles in citrated blood with the addition of microbes, as has been established for example for plague, by freeing bactericidal lysosomal protein promotes the extracellular destruction of bacteria. Immunization brings increased lysability of neutrophiles and the secretion of lysosome from them. (6). A decrease in the absorption index in our experiments coincided with the appearance in rat blood serum of antibodies for tularemia microbes in 1:10 - 1:320 titres and transition of the

infection to a phase of extinction (10). This means that the decrease in the index may have been the result of increased digestion of tularemic bacteria an accelerated phagolysis, and not a decrease in absorption activity of cells. At this time we noted a decrease in intensity of insemination of the organs by the stimulant in infected rats and transition of the infection to a phase of extinction (10).

In contrast to mice neutrophiles in rats contained degenerative changes only during the first day of infection and only after a 2 hours contact with microbes. On subsequent days, along with substantial phagolysis, the smears contained a large number of neutrophiles without degenerative changes in the nucleus, but containing large vacuoli with tularemic bacteria. By the 7th - 8th-days and subsequent to that, the neutrophiles normalized -- vacuoli are small and the granulation in clearly expressed. At this point the smears showed a significant decrease in the quantity of tularemic bacteria, freely floating and linked to the thrombocytes.

We observed adhesion of bacteria to thrombocytes among test animals, and during formulation of OCR with other stimulants such as listeria, salmonella, pseudotuber-

culous bacteria. This phenomenon was described in 1917 by Rikenberg (8), during parasitic infections. Subsequently the reaction was also traced during bacterial infection. Some investigators assess the accumulation of blood platelets and bacteria as the first phase of phagocytosis (5). According to current concepts, the aggregate of thrombocytes, bacteria, and leucocytes forms in the organism under the influence of immune processes with participation of a complex of antigens -- antibodies (9).

CONCLUSIONS

1. The absorption ability of phagocytic cells among intact animals varies within a broad range, nor reflecting the degree of infectious sensitivity to tularemia.

2. In the process of the development of the infection the index of absorption during a 30-minute exposure ti the reaction increases among the mice and rats reaching a maximum during the 5th - 6th days following infection and ending with the death of the mice. Among rats the index of absorption decreases by the 7th day and by the 15th to the 20th days corresponds to the index for the intact animals.

3. Tangible differences in the digestive activity of neutrophiles among mice and rats are noted by the second day

following infection. Among albino mice phagocytosis displays an incompleted feature, while among rats we noted over a period of 4-20 days following infection, conclusion of phagocytosis and a reduction in the number of free bacteria in citrate blood.

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ТАБЛИЦА 1. ОФР с тулярем[illegible]йным микробом у интактных животных

(1) Вид животных	8 Число опытов	9 Число животных	10 Индекс поглощения				15 Отношение индексов (60/30)
			11 через 30 мин		12 через 60 мин		
			13 Колебания показателей	14 среднее	13 колебания показателей	14 среднее	
Белые мыши (2)	4	19	4—13,2	8,2	11,7—28,7	19,9	2,4
Полевки обыкновенные 3 4	2	8	4,5—6	5,0	14,3—16	15,1	2,8
Золотистые хомяки	1	3	3—6	4	14—16	15	3,7
Морские свинки 5	5	19	3—12,8	7,7	11,6—25,6	17,2	2,2
Белые крысы 6	3	12	6—21,3	11,3	15,2—21,6	18	1,5
Кролики 7	7	19	4—20	12,4	4—25	13,8	1,1

Key to Table 1

OCR with T laremic Microbes Among Intact Animals

1. Species of Animal
2. Albinc Mice
3. Field Mouse
4. Golden Hamster
5. Guinea Pig
6. Albino Rat
7. Rabbit
8. No. of Experiments
9. No. of Animals
10. Absorption Index
11. After 30 mins
12. After 60 mins
13. Index Vaccilation
14. Average
15. Index Relationship

ТАБЛИЦА. 2 Динамика ОФР по дням после заражения

(1) Объект исследования	(5) Продолжительность реакции (мин)	(9) День после заражения: 1-й		2-й		4-й		5-й		7-й		8-й		15-й		20-й	
		индекс поглощения	% активных фагоцитов	индекс поглощения	% активных фагоцитов	индекс поглощения	% активных фагоцитов	индекс поглощения	% активных фагоцитов	индекс поглощения	% активных фагоцитов	индекс поглощения	% активных фагоцитов	индекс поглощения	% активных фагоцитов	индекс поглощения	% активных фагоцитов
(2) Зараженные мыши	30	3	12	5	20	8,7	35	14,2	67	(10) Погибли							
	60	8	32	12	49	9,7	39	26,5	83								
	120	12,6	46	17,7	71	19,2	77	22,2	89								
	Отношение индексов: 60/30 (6)		2,6		2,4		1,1		1,8	—		—		—		—	
	120/30		4,1		3,5		2,2		1,5								
(3) Зараженные крысы	30	5,7	23	12	51	21,7	83	22,2	81	14	57	9,2	37	6	24	6	24
	60	17	67	13	54	20,5	81	20,2	77	10	40	6,2	25	8,5	34	5	20
	120	14,7	59	15,5	62	10,7	43	15,2	61	9,5	38	6,7	27	3,7	15	3,2	13
	Отношение индексов: 60/30 (7)		2,9		1,08		0,9		0,9	0,7		0,6		1,4		0,8	
	120/30		2,5		1,2		0,4		0,6	0,6		0,6		0,6		0,5	
(4) Интактные крысы	30	6	24	—	—	—	—	—	—	—	—	—	—	7,5	30	—	—
	60	17,3	62											15,2	61		
	120	19	72											20,2	83		
	Отношение индексов: 60/30 (8)		2,8		—		—		—	—		—		2,0		—	
	120/30		3,1											2,5			

(For Key to Table 2, please see next page)

Key to Table 2

OCR Dynamics According to Days Following Infection

1. Subject
2. Infected Mice
3. Infected Rats
4. Intact Rats
5. Duration of Reaction in mins
6. Index Relationship
7. Absorption Index
8. % of Active Phagocytes
9. Day Following Infection
10. Deaths